Technical Bulletin 71 September 1930

University of Minnesota
Agricultural Experiment Station

Factors that Influence the Development and Survival of the Ova of an Ascarid Roundworm Toxocara Canis (Werner, 1782) Stiles, 1905, Under Field Conditions

William B. Owen
Division of Entomology and Economic Zoology

UNIVERSITY FARM, ST. PAUL

University of Minnesota
Agricultural Experiment Station

Factors that Influence the Development and Survival of the Ova of an Ascarid Roundworm Toxocara Canis (Werner, 1782) Stiles, 1905, Under Field Conditions

William B. Owen
Division of Entomology and Economic Zoology

UNIVERSITY FARM, ST. PAUL

FACTORS THAT INFLUENCE THE DEVELOPMENT AND SURVIVAL OF THE OVA OF AN ASCARID ROUNDWORM TOXOCARA CANIS (WERNER, 1782) STILES, 1905[1] UNDER FIELD CONDITIONS

WILLIAM B. OWEN

INTRODUCTION

Parasitic infections of domesticated animals are a problem of much economic importance, but information concerning infections arising from parasites lodged in the soil is meager and generally derived in a large part from the study of the parasites of man. With the growing importance of the fur-farming industry in this country there has arisen a demand for more exact information regarding the species affecting foxes and other animals that are reared under pen conditions.

Among the most common of the parasitic worms affecting foxes and other carnivors are the ascarid roundworms. Those found in foxes are very closely related to, and in most cases identical with, *Toxocara canis* of the dog. They have long been recognized as a source of loss to breeders, and as particularly dangerous to young animals. The more modern work has shown that the early larval stages undergo a complicated migration through the tissues of the animal and that in this period they may cause the death of their host in a hitherto unsuspected way.

As is well known, new infections under natural conditions result from the animal swallowing ova which have been incubated in the soil or water. A practical question often asked by fur farmers is regarding the length of time that the ova of the worms may be expected to remain viable in the soil of animal pens. It is obviously important to determine what factor or factors govern development and survival under these conditions.

To give a satisfactory answer to this question, based upon careful observations, the author carried on the studies here described. The studies on survival of ova during the winter months in the region of

[1] The writer wishes to express his sincerest appreciation to Dr. William A. Riley, Division of Entomology and Economic Zoology, for his helpful suggestions and encouragement during the course of the studies herein described. Acknowledgment is also made to Dr. Royal N. Chapman and to Mr. Allan Mail, of the Division of Entomology and Economic Zoology for placing at the writer's disposal various equipment and the thermocouple data on soil temperature.

Minneapolis, Minnesota, were supplemented with some tests on survival at low temperature under laboratory conditions. The studies on survival under summer conditions were extended to include a single experiment carried out in western Kentucky. Some data on development in air of known moisture content are also given.

ECONOMIC ASPECTS OF THE PROBLEM

The longevity of the ova of the ascarid worms in the soil is not only of biological interest but has a definite practical bearing on the problems of incidence of parasitism and renewed infection after anthelminthic treatment. Animal breeders frequently find that shortly after anthelminthic treatment their animals again become heavily parasitized. In fact, if soil where animals run, floors of pens, and bedding are contaminated with the infective ova of the worms, administration of anthelminthics can give only temporary relief. Thus far in prophylactic studies, a satisfactory method for sterilizing soil on a large scale in pens and pastures has not been found. The opinion is frequently held by many breeders of foxes that the low temperatures during the winter months in our northern states is severe enough to kill the ova that are exposed on the floors of animal pens. One phase of the present problem has been to test the validity of this much advocated idea, for, if it is true, an obvious and practical method of making pens safe would be to remove animals in the fall so as to prevent daily accession of ova and allow those present to be killed by the unfavorable weather conditions. If it is possible to establish a criterion for the survival of the ova of the ascarid worms under field conditions during the different seasons of the year, then a step will have been made toward the solution of the problem of soil infections in these forms.

HISTORICAL ACCOUNT

In the works of early helminthologists, frequent reference is made to the longevity of ascarid ova. Verloren (1854) kept the ova of *Ascaris marginata* (*Toxocara canis*) in water for more than a year and yet they were alive. Richter, as reported by Küchenmeister (1857), placed the ova of this species in water and found that they contained living embryos eleven months later. Davaine (1863) reports that the ova of *Ascaris lumbricoides* were alive after being stored for a period of five years. None of these writers give exact details as to the conditions under which the tests were made. These experiments are not strictly comparable with the studies reported here, yet they do emphasize the fact that ascarid ova may remain alive under certain conditions for one year or more.

Studies on related species, which have a more direct bearing on the present problem, are to be found only in recent literature. Yoshida (1920) placed the ova of *Ascaris lumbricoides* on the surface of the soil and under a thin layer of soil and allowed them to remain during the winter months at Osaka, Japan. Experimental feeding of these ova to a guinea pig at the end of this period proved that they were alive. Martin (1922) reports that the ova of *Ascaris suum* survived in the soil during the winter months in Nebraska. McCulloch, Graham, and Carroll (1927) failed to find viable *Ascaris suum* ova after eight months' exposure in the soil at Urbana, Illinois. On the other hand, Raffensperger (1927) reports that in one experiment the ova of this same species survived for one year under field conditions at Chicago, Illinois. None of the above mentioned writers have given actual soil temperature records for the period of exposure.

More critical studies on this problem, which take into consideration the different environmental factors that may influence development and survival of ascarid ova, are as follows: Brown (1927) found that the development and survival of the ova of *Ascaris lumbricoides* under field conditions in Panama were governed by the type of soil in which exposed and whether they received full sunlight or were in the shade for the period of exposure. Caldwell and Caldwell (1928) report experiments carried on in Alabama during the month of July, in which the ova of *Ascaris lumbricoides,* in fecal plants on different types of soil, disintegrated on three days of exposure to full sunlight. Ova in like cultures, but placed under shade conditions, were relatively unaffected when examined more than a month later. Viable ova were recovered from soil cultures which had been under natural conditions from August until the following May. Brown (1928) reports that the ova of *Ascaris lumbricoides* and *Ascaris suum* survived in sand cultures under weather conditions at Baltimore, Maryland, from December 30 to May 2. Riley and Owen (1928) and also Owen (1928, preliminary report) found that the ova of *Toxocara canis* survived in the soil under field conditions in the region of Minneapolis, Minnesota, from November 11 to April 26.

MATERIALS AND METHODS

Toxocara canis was selected as the object of these studies because of its high incidence and close relationship to some of the other roundworms found in a number of domesticated carnivors. It is a common parasite of dogs, which afford a convenient source for the collection of the adult worms.

In planning each experiment, attempts were made to expose the ova in outdoor cultures which would be comparable with conditions they

would normally encounter in nature. The ova were collected from the uteri of gravid females. Only those ova from the terminal portion of the uteri that were known to be fully mature were used. The culture plots in which they were planted were prepared from different types of soil—sand, clay, and humus. Small flower pots, 3:5 inches in diameter at the top and 4 inches in depth, were filled with the desired type of soil and sunk into the ground to such a depth that the surface of the soil in the container was on the same level as that of the surrounding ground. The ova were placed on the surface of the soil in the container or mixed with the upper half inch of soil stratum, depending on the condition desired. No exact estimate of the number of ova planted in each culture was made; however, an approximate estimate would be about 75,000. In every experiment performed, a number of the ova were cultured at 25 degrees centigrade as a check to determine whether they were fertile.

The records of soil temperature during the winter months were obtained with a standard copper-constantan thermocouple. The winter cultures were placed about eight inches from the instrument. The soil temperature records for the summer cultures were obtained with a mercury thermometer.

To facilitate the making of differential counts after ova were recovered from the soil, the isolation method used by Caldwell and Caldwell (1928) and in part, that described by Spindler (1929), was used. This method briefly consists of (1) treating a soil sample containing ova with a 30 per cent antiformin solution to free them from soil particles, and (2) floating the ova from the material with a solution of high specific gravity, as concentrated sugar solution or sodium dichromate. In these studies sodium dichromate was used to float the ova from a treated soil sample. The surface film of this mixture is looped off with the open end of a small test tube, transferred to a glass slide, and examined with a 16 mm. objective. In the tables of results, the ova are classified upon the basis of their developmental progress and state, i.e., disintegrated, morula, motile embryos. After certain experiments, infection tests were made on guinea pigs and laboratory rats to determine the viability of the motile embryo state.

SURVIVAL UNDER WINTER CONDITIONS IN MINNESOTA[2]

To obtain the data on survival of ova under winter conditions in Minnesota, three cultures, using sand, clay, and humus soil as media, were prepared on November 11, 1927, and placed for exposure on University Farm. The ova in an unsegmented state were mixed with

[2] A brief report of this experiment was published by Riley and Owen (1928) and also by Owen (1928).

the upper half-inch soil stratum in each culture, thus making the conditions comparable with those to which they would normally be subjected in nature. On April 26, 1928, the cultures were brought into the laboratory for observation. At this time, a number of the ova were isolated from a soil sample. None showed further development. Obviously, because of the low temperature they had remained dormant during the entire period of exposure. The cultures were then placed in an incubator, which was maintained at 25° C. until May 12, when the ova were isolated and final differential counts were made. The results of this experiment are given in Table I.

TABLE I

DEVELOPMENT SHOWN BY THE OVA OF *Toxocara canis* AFTER EXPOSURE TO FIELD CONDITIONS FROM NOVEMBER 11, 1927, TO APRIL 26, 1928

Culture	Number counted	Undeveloped	Morula*	Vermiform	Motile embryos	Dead
		per cent	per cent	per cent	per cent	per cent
Humus	200	13.0	4.5	6.0	74.0	2.5
Clay	200	4.5	0.5	11.0	79.0	5.0
Sand	200	4.5	5.0	8.5	80.0	2.0

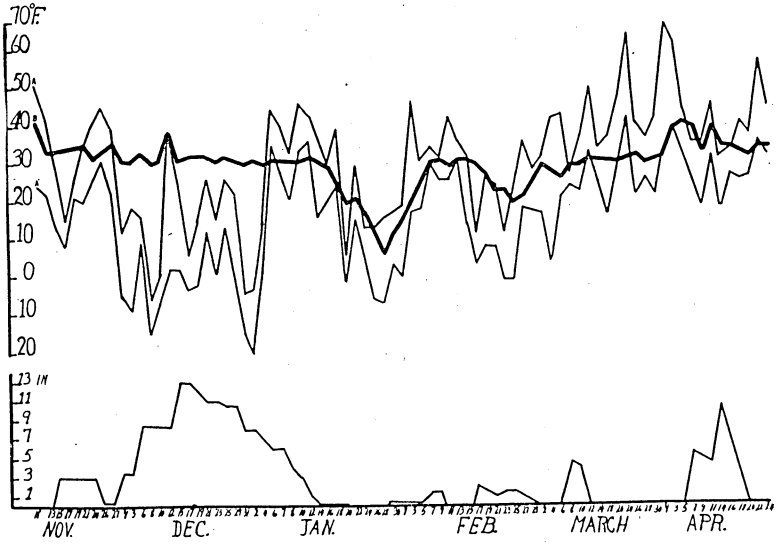
* For convenience, the term morula is used in this report to designate all multicellular developmental phases previous to the vermiform stage.

Consideration of these data shows that from 74 to 80 per cent of the ova developed motile embryos with only sixteen days' incubation, after the cultures were placed at a temperature of 25° C. From 84 to 93 per cent were viable, as is shown by the proportion in developmental stages at the time the counts were made. These data coincide very closely in all the cultures. The differences are probably owing to the technic of recovering ova from the soil rather than to any influence the type of soil exerted.

To test whether these motile larvae were reduced in vitality by the ova having been subjected to the temperature indicated in Graph 1 and remaining dormant in the unsegmented condition for more than five months previous to development, about 500 were fed to a young laboratory rat. At the expiration of three days, the lungs and liver were examined with a Baermann isolating apparatus for migrating larvae. Four larvae were recovered. This number seems small, but when compared with the results of Scott (1928) on recovering hookworm larvae from the organs of the host by the same method, it is significant.

Graph 1 indicates the physical conditions of the environment during the course of the experiment. The soil temperature readings were taken on alternate days. The air temperature curves were plotted from readings taken on the days the soil temperature was recorded. Graph 1 illustrates some significant points to be taken into consideration in a study of field conditions during the winter months in any of the north-

ern states. Snow and, to a less extent, ice serve as a blanket to keep the superficial soil temperature from fluctuating as does the air temperature. Records show that the weather conditions of December were the most severe in years, having 22.8 inches of unmelted snowfall and a mean air temperature of 7.34° F. (—13.7° C.). The lowest recorded air temperature for December was —15° F. (—26.11° C.). On the day this record was obtained the soil temperature under 5.5 inches of



Graph 1. Air Temperature, Soil Temperature, and Snowfall from November 11, 1927 to April 24, 1928

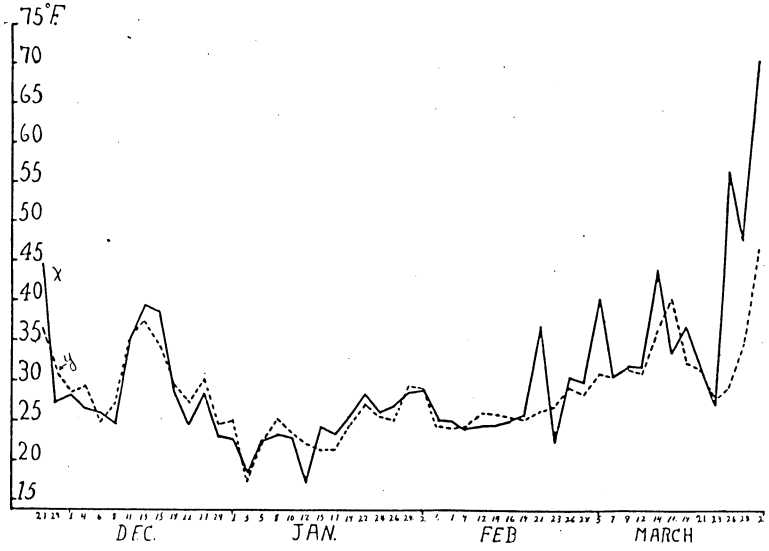
Explanation: A, maximum air temperature; A', minimum air temperature; and B, soil temperature at two inches beneath the surface. (No provision for surface soil temperature records was made during this season.) Snowfall is shown in inches. The air temperature data are from the United States Weather Bureau, Minneapolis station. The soil temperature data are thermocouple readings taken at site of the cultures. The snowfall was likewise measured at site of cultures.

snow was 31.64° F. (—0.23° C.). The lowest recorded air temperature for the entire period was —20.0° F. (—28.89° C.). On this date the soil temperature under 5 inches of snow was again 31.64° F. (—0.23° C.). The lowest recorded soil temperature during the entire period was 6.26° F. (—14.33° C.). The air temperature on this date was —6.0° F. (—21.11° C.). These data clearly demonstrate that in a region of much snowfall, air temperatures are not a true index to the temperature conditions on the surface of the soil.

During the winter of 1928 and 1929, soil temperature records were kept with the same apparatus, to which was added a surface junction. The records of this season are given in Graph 2.

Comparison of the figures from the surface with those from two inches beneath the surface indicate that there was little difference in the temperature at the two levels. It is interesting to note that the lowest temperature recorded on the surface of the soil during the winter of 1928-29 was 17.24° F. (—8.2° C.). Records of soil temperature during the winter months of two consecutive years show that at no time did it become cold enough to kill the ova of this species, as shall be seen from laboratory tests on viability at low temperatures.

Data from current studies on the resistance of helminth eggs to weather conditions do not as a rule take into consideration the striking contrast between air and soil temperatures during the season of heavy



Graph 2. Soil Temperature Records from November 27, 1928 to April 2, 1929
Explanation: X, soil temperature at the surface; Y, soil temperature at two inches beneath the surface. Data taken from thermocouple readings.

snows. It is obvious from the above data that air temperature readings alone, when considered apart from other physical factors, can not be relied upon during the winter months to give a true picture of conditions on the surface of the soil.

DEVELOPMENT AND SURVIVAL UNDER SUMMER CONDITIONS

In Minnesota

The studies on the development and survival of the ova of this species under summer conditions in Minnesota were begun during the season of 1928. On May 11 of that year, ova in an unsegmented condi-

tion were placed on the surface of cultures that were prepared from sand, clay, and humus soil, respectively. As these ova were to remain under weather conditions during the summer months, it was thought that the beating rains and activities of soil organisms would be sufficient to cover them. The cultures were placed on the University Farm in such a position that they received full sunlight at the beginning of the period of exposure. During the summer, however, grass grew around the cultures so that for the latter part of the season they were somewhat protected from the sun. Unfortunately, the author was not able to keep records of the soil temperature during the experiment, or to make examination of the ova at desired intervals. Examination of the ova on October 13, 1928, showed that they had all disintegrated. Only 35 ova were recovered from the three cultures. The contents of these were hyaline, and many contained a large refractive bubble which filled much of the interior of the ovum. Brown (1928) reports finding bubbles of like nature in the ova of *Ascaris lumbricoides* which had broken down as a result of dessication.

A similar study was made during the summer of 1929. Soil cultures of sand and humus were prepared and exposed under three conditions of sunlight. One series was placed in full sunlight; another, in thin grass about 14 inches high, so that it received some shade; and a third, under a hedge where there was complete shade. Unsegmented ova were planted on the surface of these cultures on July 5. There occurred almost 2 inches of precipitation on July 6 that mixed the ova with the surface soil particles and gave conditions comparable with those in animal pens of dirt floors. Soil temperature records and relative humidity readings of the air above the cultures were taken in the early morning before the sun had shone on them and again at 1:30 p.m., which is perhaps the hottest part of the day in this region. These data are recorded in Graph 4. The results of the examination of the ova at definite intervals are given in Table II.

Consideration of Table II, which gives the developmental stages of the ova at the intervals of examination, shows that in full sunlight none developed beyond the morula stage and that all were dead at the expiration of 11 days. Some of the ova exposed to full sunlight did reach the late morula stage but none in either the sand or humus culture developed so far as the vermiform stage. With some shade, the maximum number found in the motile embryo stage was 53.5 per cent. This was in the humus culture, as compared to only 18 per cent in the sand culture. In the shade cultures, 75 per cent of the ova in the humus soil were in the motile embryo stage when last examined, whereas in the sand soil, only 45 per cent were in this stage.

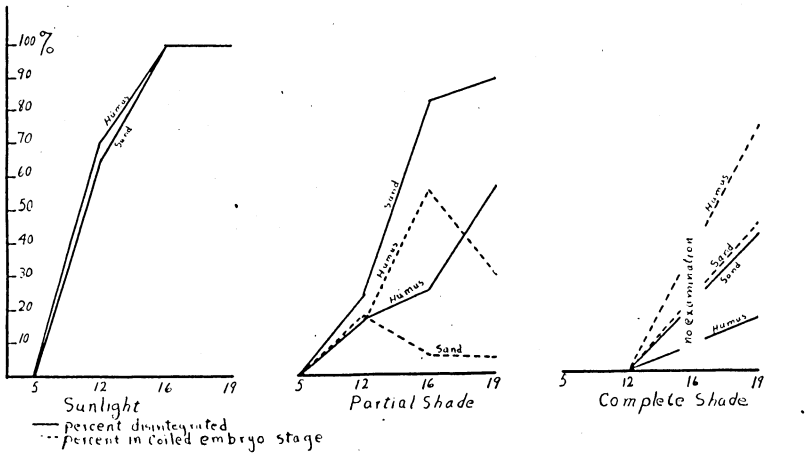
TABLE II

DEVELOPMENT AND SURVIVAL OF THE OVA OF *Toxocara canis* EXPOSED IN SAND AND HUMUS SOILS UNDER THREE CONDITIONS OF SUNLIGHT, FROM JULY 5 TO JULY 19, 1929 AT MINNEAPOLIS, MINNESOTA

	Sand						Humus					
Date examined	No. counted	Undeveloped	Morula	Vermiform	Motile embryos	Dead	No. counted	Undeveloped	Morula	Vermiform	Motile embryos	Dead
		per cent	per cent	per cent	per cent	per cent		per cent	per cent	per cent	per cent	per cent
						Sunlight						
7/12	200	1.0	35.0	0	0	64.0	200	0	30.0	0	0	70.0
7/16	200	0	0	0	0	100.0	200	0	0	0	0	100.0
7/19*												
						Part Shade						
7/12	200	11.5	28.5	18.0	18.0	23.5	200	15.5	23.0	27.0	17.0	17.5
7/16	200	5.0	2.5	4.5	5.5	82.5	200	17.5	1.0	2.5	53.5	25.5
7/19	200	3.5	1.5	0	5.0	90.0	200	11.5	0	1.0	30.0	57.5
						Complete Shade						
7/12	200	18.5	82.5	0	0	0	200	18.66	81.34	0	0	0
7/16*												
7/19	200	7.5	2.5	2.5	45.0	42.5	200	7.5	0	0	75.0	17.5

* No examination.

Graph 3 gives a comparative picture of the number of ova disintegrating in the two types of soil under the three conditions of sunlight. In like manner, the survival of ova reaching the motile embryo stage under part shade is illustrated very vividly in the graph. The number of ova in this stage reached a maximum (18 per cent) in the sand culture on July 12 (7 days' exposure) then dropped to 5.5 per cent on July 16 (11 days' exposure). In the humus culture, the per cent developing to the coiled embryo stage reached a maximum (53.5 per cent) on July 16 (11 days' exposure), then dropped to 30 per cent on July 19 (14 days' exposure). Observations on the ova under complete shade were not carried far enough to designate the exact time at which the greatest number were in the coiled embryo stage. However, by looking at Table II, it is evident that the percentages found in that

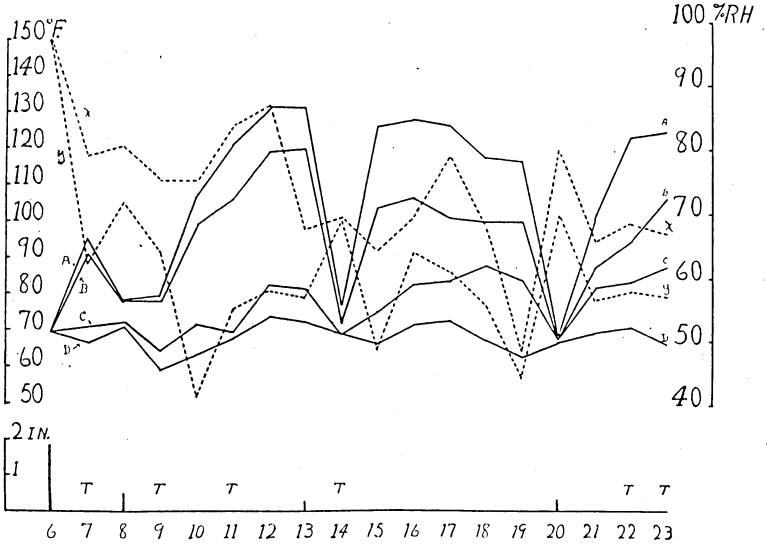


Graph 3. .Disintegration and Survival of Ova in Sand and Humus Soils Under Three Conditions of Sunlight from July 5 to July 19, 1929. . Data taken from Table II.

stage on July 19 (14 days' exposure), were very close to a maximum. From the data given in Graph 3 on disintegration and survival under part shade, it is possible to compute the percentage of ova disintegrating in the two types of soil before reaching the coiled embryo stage. This number is arrived at by subtracting the percentage of ova disintegrating after reaching the coiled embryo stage from the final disintegration figure. In the sand culture, 90 per cent — (18—5) = 77 per cent, the approximate number disintegrating before reaching the coiled embryo stage. In the humus culture, 57.5 per cent — (53.5—30) = 34 per cent, the approximate number disintegrating before reaching the coiled embryo stage. It is evident, from the data in Graph 3, that disintegration was much more rapid in sand soil than in humus under both part shade and complete shade. In full sunlight under the conditions of

this experiment, the rate of disintegration was approximately the same in both types of soil.

Graph 4 gives the soil temperature records and relative humidity readings under the three conditions of sunlight during the experiment. The variation in relative humidity was so small under the three conditions that the complete series of readings has been combined into one graph. It should be remembered, however, that this graph does not give the relative humidity of the air among the soil particles on the surface, when the temperature at this level is higher than that of the air above. No measurement of the intensity of the light was made. However, by comparing the surface-soil temperature records, it is evident that there was a gradient in respect to the amount of light received under



Graph 4. Soil Temperature, Relative Humidity (Taken at a Level Two Feet Above the Soil), and Precipitation from July 6 to July 23, 1929. Data Recorded at Minneapolis, Minnesota

Explanation: A, maximum soil temperature in full sunlight; B, maximum soil temperature in part shade; C, maximum soil temperature in complete shade; D, minimum soil temperature; X, maximum relative humidity; Y, minimum relative humidity. Precipitation expressed in inches. T, traces of precipitation.

the different conditions. The highest recorded soil temperature in full sunlight was 131.9° F. (55.5° C.), whereas the highest temperature in part shade was 119.3° F. (48.5° C.). In complete shade, the temperature did not go above 84.2° F. (29.0° C.). The difference between sand and humus soil under like conditions was never more than a few degrees. The data given in Graph 4 indicate that high temperature alone, when considered separately from the dessicating effect of heat combined with dryness, was a factor in the destruction of ova under

conditions of full sunlight. This is reasonable, as the surface-soil temperature in full sunlight on two consecutive days reached 131.9° F. (55.5° C.). This reading, it should be remembered, is not taken from a constant temperature record throughout the day. Thus it is very probable that this figure is not the highest that could be obtained for maximum soil temperature under these conditions.

In Kentucky

In the latter part of July and in August of 1928 an experiment was carried out in the western part of Kentucky to get data on the development and survival of ova under summer conditions in that region. Cultures, in series of two, were prepared from sand, clay, and humus soil, and exposed under two conditions of sunlight. One series received full sunlight and the other was located in the shade of a walnut tree. Unsegmented ova were placed on the surface of these cultures on July 23, 1928. In order to duplicate conditions found in a fresh stool of an animal, these cultures were moistened at the time the ova were planted. The surface of the soil in the series receiving full sunlight became dry with one day of exposure, whereas the cultures in the shade remained moist for many days.

Examination of the ova in the series exposed to full sunlight on August 6 (14 days' exposure), showed that they were all dead. Many of these contained the large bubble which was found in disintegrated ova after being exposed to summer conditions in Minnesota. The ova exposed in cultures under shade conditions were examined at intervals as indicated in Table III.

TABLE III

DEVELOPMENT SHOWN BY THE OVA OF *Toxocara canis* IN CULTURES UNDER SHADE CONDITIONS—EXPERIMENT STARTED JULY 23, 1928

Culture	Date examined	No. counted	Undeveloped	Morula	Vermiform	Motile embryos	Dead
			per cent	per cent	per cent	per cent	per cent
Sand.....	Aug. 13	200	1.0	1.5	0.0	90.0	7.5
Sand.....	Sept. 3	100	0.0	1.0	0.0	93.0	6.0
Clay.....	Aug. 13	200	3.5	0.0	0.0	90.0	6.5
Clay.....	Sept. 3	100	0.0	0.0	0.0	94.0	6.0
Humus.....	Aug. 13	65*	4.6	0.0	0.0	86.6	8.8
Humus.....	Sept. 3	†					

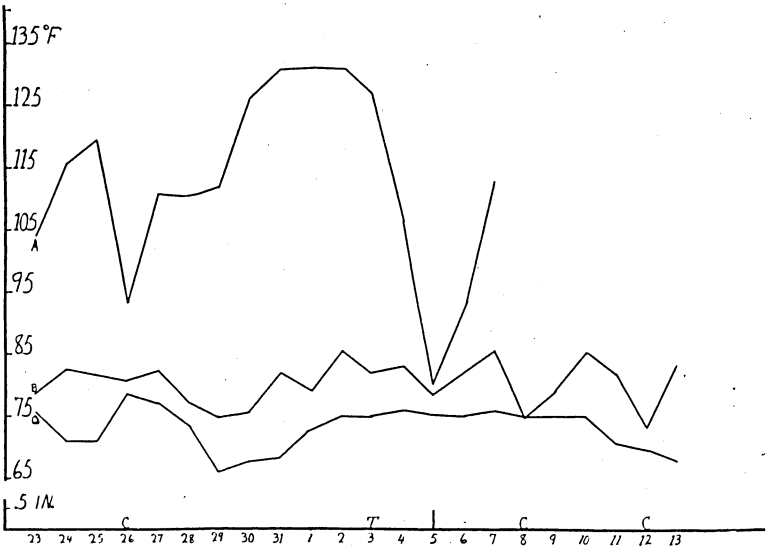
* This figure represents the total number of ova recovered from the entire culture. An error in the original number planted perhaps accounts for this result.

† No examination.

The data given in Table III indicate that the amount of disintegration did not increase in sand and clay soil from August 13 (21 days' exposure) to September 3 (42 days' exposure). During this period the number in the coiled embryo stage increased a little, as is shown by the records from sand and clay cultures.

Graph 5 indicates the physical data recorded along with this experiment. The great contrast between the surface-soil temperature in shade and in full sunlight is again seen here. In full sunlight, the highest recorded soil temperature was 131.9° F. (55.5° C.), whereas the highest temperature found in the shade cultures was 87.8° F. (31.0° C.).

That ova in the series exposed to full sunlight should disintegrate with 14 days' exposure in the soil is not surprising, as the temperature on three consecutive days reached 131.9° F. (55.5° C.). Here, as was found under like conditions in Minnesota, the surface-soil temperature reached the absolute maximum or lethal point for the ova of this species. Examination of these ova at an earlier date would have given more accurate information on the actual time they will survive under the conditions here tested, but it was impossible to make such an observation.



Graph 5. Soil Temperature and Precipitation from July 23 to August 13, 1928
Data Recorded in Western Kentucky

Explanation: A, maximum soil temperature in full sunlight; B, maximum soil temperature in shade; O, minimum soil temperature. Precipitation given in inches. C, cloudy days. T, traces of precipitation. All data recorded at site of cultures.

Longevity in the Soil

The results of a single experiment on the longevity of the ova of *Toxocara canis* in the soil in the region of Minneapolis, Minnesota, are as follows. On November 27, 1927, ova in an unsegmented state were planted in a culture of humus soil and mixed with the upper half-inch soil stratum of the same. A soil sample of this culture was brought into the laboratory on February 4, 1928, and placed in an incubator that was kept at 25° C. On February 18, the ova were recovered

and differential counts made. The results of this examination are given in Table IV, which shows that 84.5 per cent contained motile embryos and only 2.5 per cent had disintegrated.

TABLE IV

DEVELOPMENT OF THE OVA OF *Toxocara canis* AFTER BEING IN THE SOIL FROM NOVEMBER 24, 1927 TO FEBRUARY 4, 1928

	Undeveloped	Morula	Vermiform	Motile	Embryos	Dead
	per cent	per cent	per cent	per cent	per cent	per cent
200.....	11.0	1.5	0.5	84.5	...	2.5

The ova in this experiment were not examined again until November 24, 1928, which was one year from the date they were placed in the soil. In this examination, a total of 55 ova were recovered from the entire culture. Of this number, 22 were hyaline, 22 contained a large bubble, and in 11 the outlines of the embryos were visible but disintegration was in progress.

This culture was located close enough to the thermocouple that Graph 1 expresses the physical condition of the environment during the winter months. The culture received full sunlight during the early summer but was protected from the heat by grass during the hottest part of the summer. The fact that the embryos in some of these ova had not completely degenerated when last examined, suggests that the ova of *Toxocara canis,* covered with surface soil to the depth that these were, may survive the summer months or even for one year under the conditions of this experiment in Minnesota.

DEVELOPMENT AND VIABILITY AT LOW TEMPERATURES UNDER LABORATORY CONDITIONS

In this study the ova were collected from the uteri of gravid females and kept in a 2 per cent formalin solution as a culture medium. The cultures were exposed in low temperature cabinets for varying lengths of time, as indicated in the summary of these studies given in Table V.

Ova from cultures numbers 7 and 8 were fed to young laboratory rats to determine whether they were capable of infecting an animal after being exposed to the temperature given in Table V. No migrating larvae were recovered from the organs of these test animals. This fact, with the further observation that these larvae disintegrated after an elapse of one week, indicates that —25.6° F. is fatal to infective ova of this species. The data in Table V indicate that 53.6° F. is very close to the minimum effective temperature for unsegmented ova, as no development occurred here during a period of 35 days. On the other hand, ova exposed at 62.0° F. for the same length of time, developed to the coiled embryo stage. The data obtained in these experiments compare

very favorably with similar studies made by Cram (1924) on the ova of *Ascaris lumbricoides*.

TABLE V

SUMMARY OF STUDIES ON DEVELOPMENT AND VIABILITY OF THE OVA OF *Toxocara canis* AT LOW TEMPERATURES UNDER LABORATORY CONDITIONS

Condition of ova	Temperature	Period of exposure	Condition of ova	Result when cultured at 77.0° F.
1. Unsegmented	Constant at 62.0° F.	35 days	35 per cent motile embryos	Normal development
2. Unsegmented	Constant at 53.6° F.	35 days	No development	Normal development
3. Unsegmented	5 to −31.0° F.	7 days	No development	No development
4. Unsegmented	Alternated between 77.0° F. and 5.0 to −31° F.	3 days at each temp. for 15 days	No development	Died in 16-cell stage
5. Motile embryos	Constant at −13.0° F.	8 hours	Motile	Motile
6. Motile embryos	Constant at −13.0° F.	24 hours	Motile	Motile
7. Motile embryos	Constant at −25.6° F.	8 hours	Very little movement	Disintegrated in 7 days
8. Motile embryos	Constant at −25.6° F.	30 hours	None motile	Disintegrated in 7 days

DEVELOPMENT AND VIABILITY IN AIR OF KNOWN MOISTURE CONTENT

The data given in this report, on development and viability in air of known moisture content, are preliminary to further studies by the author on the moisture requirements of the ova of *Toxocara canis*. The moisture content of the air in these experiments was controlled by the chemical method. During the period of exposure the ova were in small, open vials 1 inch deep, over which air of the desired moisture content circulated. A summary of these studies is given in Table VI.

From the data given in Table VI, it is obvious that loss of water retards development. This corroborates the results of Otto (1929) on culturing the ova of different ascarids in air of known moisture content. Unless dessication is complete, development is resumed if the ova are returned to a water medium. This fact is illustrated by the figures on development at 52 per cent relative humidity. In air of 52 per cent relative humidity for a period of 7 days, the limit of development was the morula stage, with 87 per cent in this condition. These ova were then returned to a water medium, under which condition 78 per cent developed to the coiled embryo stage. In air of 52 per cent relative humidity, for a period of 11 days, 92 per cent of the ova were in the morula stage, but when transferred to a water medium only 4 per cent developed to the coiled embryo stage. At a temperature of from 27° to 30° C., the minimum requirement for ova to develop to the

TABLE VI

DEVELOPMENT AND VIABILITY SHOWN BY THE OVA OF *Toxocara canis* WHEN CULTURED IN AIR OF VARIOUS PERCENTAGES OF RELATIVE HUMIDITY*

A

Ova in unsegmented stage when exposed to humidified air

Days exposure	Relative humidity	Maximum development in humidified air	Motile	Disintegrated	Development when returned to water medium
	per cent		per cent	per cent	
3	0.0	7% early morula	...	89.0	No development
7	0.0	None	...	100.0	No development
3	31.0	17% morula	...	3.0	Continued development
7	31.0	20% morula	...	67.0	No development
7	52.0	87% morula	...	8.0	78% dev. to coiled embryos
11	52.0	92% morula	...	5.0	4% dev. to coiled embryos
20	52.0	.....	...	100.0	No development
8	58.3	1.5% vermiform	...	23.5	No observations made
16	58.3	Morula (no count made)	...	(?)	8% dev. to coiled embryos
8	70.4	4% vermiform	...	4.0	80% dev. to coiled embryos
16	70.4	Morula (no count made)	...	(?)	30% dev. to coiled embryos
7	75.0	7.5% coiled embryos	7.5	4.0	77% dev. to coiled embryos
20	75.0	81% coiled embryos	3.0	14.0	Larvae freed from shell in contact with water 64% motile
7	nearly saturated	84.5% coiled embryos	84.5	0.0	

B

Ova cultured to motile embryo stage previous to exposure

Days exposure	Relative humidity	Per cent motile after exposure	Per cent disintegrated	Per cent of larvae motile when freed in water
1	0.0	80.0	7.0	
2	0.0	66.0	32.0	
7	31.0	0.0		20.0
20	52.0	0.0		1.0
20	75.0	57.0	38.0	
20	nearly saturated	83.7	2.5	

* The temperature ranged during the period of exposure from 27 to 30° C.

coiled embryo stage is a relative humidity of from 70 to 75 per cent. At 70.4 per cent relative humidity, for a period of 16 days, no ova were found beyond the morula stage, and when ova from this culture were returned to a water medium, only 30 per cent developed to the coiled embryo stage. This indicates that they were dying very rapidly under these conditions. At 75 per cent relative humidity, for a period of 20 days, 81 per cent of the ova had reached the coiled embryo stage and only 14 per cent had disintegrated. In air nearly saturated with moisture, 84.5 per cent of the ova developed to the coiled embryo stage in 7 days, which is just as rapid and within the range of the percentage which reach this stage when cultured in water or 2 per cent formalin. The maximum number of ova that undergo development when taken from the uteri of females, as these were, is from 85 to 95 per cent. A point of practical interest found in these studies is that lack of motility under the warm-stage microscope is not a sure test of whether the

larvae are alive. This fact is illustrated by the ova cultured for 20 days at 75 per cent relative humidity. Under these conditions 81 per cent were in the coiled embryo stage, but only 3 per cent showed motility under the warm stage microscope. Yet when freed from the shell and in contact with water, 64 per cent of the larvae were motile. There is some indication that ova in the coiled embryo stage have a greater resistance to dessication than when in the various stages of development. For example, 31 per cent relative humidity for a period of 7 days was lethal to developing ova, but with ova containing coiled embryos when exposed to the same conditions for the same length of time 20 per cent of the larvae were definitely alive.

GENERAL DISCUSSION

Survival of Ova Under Winter and Summer Conditions

These studies clearly showed that from 75 to 80 per cent of the ova of *Toxocara canis* can, in the unsegmented state, survive the winter months in the soil under weather conditions in the region of Minneapolis. This apparent resistance to very low temperatures is accounted for by the fact that the surface-soil temperature under the protection of heavy snows is always much higher than the air temperature. This fact does not exclude the possibility, however, that extremely low temperatures may occur at a time when there is no snow on the ground. Inasmuch as laboratory tests indicate the ova of this species are killed at −25° F., there is always the chance that under certain conditions this temperature may be reached on the surface of the soil. However, records for two successive winters show that the lowest temperature recorded on the soil surface was 6.26° F. (−14.33° C.).

Observations on ova containing motile embryos, which were exposed to −13° F. (−25° C.) under laboratory conditions, demonstrate that they are relatively unaffected by this temperature. Thus it is safe to conclude that ova containing motile embryos would have survived under field conditions during the winter months equally as well as those in an unsegmented condition.

Under conditions of low temperature, the ova of this species may remain dormant in the soil for more than five months and then resume development if the temperature is raised above that of the minimum effective point. Ova having remained dormant for this period are not greatly reduced in vitality, as is seen by the large number that developed motile larvae. The larvae developed from these ova are capable of infecting an animal, as was shown by the test on a young laboratory rat.

The evidence derived from the studies reported here on the survival of the ova of *Toxocara canis* in the soil during the summer months cor-

roborates the conclusions of Brown (1928), Caldwell and Caldwell (1928), and Otto (1929) that dessication is the greatest lethal factor to development and viability of ascarid ova under field conditions. An additional point brought out in the present studies is the fact that the surface-soil temperature in full sunlight may, during the hottest part of the summer, reach the absolute maximum temperature for ascarid ova (55.5° C.), as indicated by the records from both Kentucky and Minnesota.

Relation of Various Physical Factors to Dessication of Ova

The question of the relative suitability of different soil types for development and survival of ascarid ova under field conditions and the relation this bears to incidence and distribution, has been given considerable attention by recent investigators. Brown (1927), working at Penonome, Panama, found that the ova of *Ascaris lumbricoides* in sand cultures exposed to full sunlight disintegrated before reaching the coiled embryo stage, whereas from 85 to 90 per cent of those in clay, loam, and humus soil cultures, under like conditions became embryonated. Otto (1929) reports that in southwestern Virginia *Ascaris* ova exposed to sunlight in a mixture of cinders and loam were never more than 3 per cent embryonated. In sand and loam soils, 36 and 38 per cent and in clay a maximum of 73 per cent developed to the coiled embryo stage. Under shade conditions the maximum number found in the embryonated stage was about the same in all four types of soil, varying from 73 per cent in sand to 89 per cent in clay. In a laboratory experiment in which he passed air of different relative humidities over soil cultures of sand, loam, clay, and humus which had been previously oven dried, it was found that ova in sand and humus in air of 70 to 75 per cent relative humidity developed to the coiled embryo stage, but in air of from 40 to 50 per cent relative humidity, the ova disintegrated in all the soils before becoming embryonated. In the same experiment he used air saturated with moisture and found no difference in the number becoming embryonated in the different types of soil. At the end of the experiment, it was found that the sand had taken up less moisture in saturated air than the other soils did in air either 40 to 50 per cent saturated or 70 to 75 per cent saturated. Altho the sand contained less moisture by weight in air of 70 to 75 per cent relative humidity than the other soils did at a relative humidity of 40 to 50 per cent, ova became embryonated in sand at 70 to 75 per cent relative humidity and disintegrated in all the soil cultures at 40 to 50 per cent relative humidity. In a previous test, he had shown that ova could become embryonated in air of from 70 to 75 per cent saturated with moisture. From these data he states that "Under identical conditions of temperature and

atmospheric moisture one soil apparently makes as good a cultural media as another. . . . It is at once evident that the relative soil moisture does not affect the suitability of these soils for culturing ascarid eggs."

The results of the above experiment are what might have been expected, but do not justify the interpretation placed upon them by Otto. A fundamental principle overlooked by the investigator is that the moisture content of the air and the soil moisture at the surface tend to reach a state of equilibrium. Evaporation of moisture from the soil and the removal of moisture from the air by the soil are processes resulting from this principle. It is very evident that the ova on the surface of the sand culture, in air of from 70 to 75 per cent relative humidity, were not dependent upon soil moisture to prevent dessication. For in this test, the conditions were such that this state of equilibrium could be reached between the moisture content of the air among the soil particles on and near the surface and the air above the culture. The relative suitability of different soils as culture media for ascarid ova, as shall be discussed later, is dependent upon the texture and composition of the soils, which in turn determines the rate of evaporation, under like conditions, moisture capacity, and like characteristics.

In the present studies, ova in sand, clay, and humus soils exposed to full sunlight during the hottest part of the summer in both Kentucky and Minnesota disintegrated before developing to the coiled embryo stage. It is not clear in these experiments whether it was a combination of high temperature and lack of moisture or high temperature alone that killed the ova, as in both tests the surface soil temperature reached 131.9° F. (55.5° C.) before they were examined. Under part shade conditions in Minnesota, a maximum of 18 per cent reached the coiled embryo stage in sand, as compared with 53.5 per cent in humus. After 14 days' exposure under these conditions, only 5 per cent were left in the embryonated stage in sand in contrast to 30 per cent in humus. In this test the sand was observed to dry out more quickly than the humus, and likewise the temperature in the sand culture on three days during the first week of exposure was 5 degrees higher than in the humus. In the shade cultures in Minnesota, where the temperature was the same in both cultures but remained relatively low, development was retarded. When these ova were last examined, 75 per cent in the humus soil were embryonated and 17.5 per cent disintegrated as compared with 45 per cent embryonated and 42.5 per cent dead in the sand. In cultures of sand, clay, and humus under full shade in Kentucky, the number becoming embryonated was approximately the same in each soil type (85 to 90 per cent) and observations on ova in sand and clay cultures indicate that no disintegration occurred with 42 days' exposure during the latter part of July and the month of August.

The explanation for the higher disintegration of ova in sand as contrasted with humus soil under both part shade and complete shade in Minnesota is to be found in the nature of the soil in which they were exposed and other physical forces present during the period of exposure. The *rate* of evaporation of moisture from the soil is regulated by temperature, relative humidity, wind velocity, and size of soil particles, which determines the water-binding capacity, compactness, etc. The free water in the soil (hygroscopic not included) available for organisms is determined by the chemical nature of the soil and size of soil particles. Humus soils ordinarily contain more available moisture for organisms than do clay, sand, and others. Under field conditions, apart from very exceptional cases, evaporation from sand is more rapid than from clay and humus soils. As the soil becomes dry, the temperature rises, which in turn increases evaporation, if humidity conditions permit. In the cultures under part shade, when the soil temperature ranged from 100 to 119° F., evaporation was rapid. However, the rate of evaporation was greater from the sand than from the humus because of the larger air spaces between the sand particles, which permit better aeration at the surface. In complete shade the physical conditions were the same except for the lower surface-soil temperature, which was never more than 84.2° F. Here the rate of evaporation was slower and the disintegration of ova less than in part shade, but higher in sand than in humus soil. How then can the lack of disintegration of ova in sand and clay soils after 42 days' exposure in the shade in western Kentucky be explained? The answer to this question is that the physical factors involved in the shade experiment in Kentucky were not the same as in a similar test in Minnesota. A striking difference is the greater average wind velocity in Minnesota which would increase the rate of evaporation in cultures that were otherwise subjected to the same conditions. From this discussion it is obvious that the death of ascarid ova from dessication under field conditions is the result of the combined effect of a series of interdependent physical factors, of which soil type is one of the most important.

The results of laboratory tests on culturing the ova of *Toxocara canis* in air of different relative humidities indicate that they are relatively resistant to dessication. The data obtained in these tests suggest three important points which have bearing on the development and survival of the ova of this species under field conditions: (1) The water content of an egg at the time it is discharged does not have to be maintained during the developmental period. (2) The loss of water by evaporation up to a certain amount has no apparent harmful effects, but beyond this point ova die rapidly. (3) Ova are retarded in develop-

ment by loss of water, but unless they die of dessication, they continue development when given moisture. The slow rate of evaporation combined with this faculty of remaining alive when partly dessicated probably accounts for field observations that ova remain viable in apparently dry soil for many days. If this interpretation is correct, the amount of water lost through evaporation and the chances of coming in contact with available soil moisture are determined by the nature of the soil and the other physical factors during the period of exposure. It follows, then, that the ratio between loss and gain of water by ova is determined by the behavior of a natural physical system which varies in its effects as any one of the physical factors involved varies.

SUMMARY AND CONCLUSIONS

The ova of *Toxocara canis* survived the winter months under weather conditions during the season of 1927 and 1928 in the region of Minneapolis, Minnesota.

Observations during two consecutive winter seasons indicate that the surface-soil temperature did not go low enough to kill the ova of this species, altho air temperature was as low as —24° F. (—31.11° C.).

The great contrast between air temperature and soil temperature during the winter season is accounted for by the abundance of snow, which acts as a blanket and keeps the surface soil relatively warm.

Fur farmers can not rely upon having the soil temperature of animal pens in which snow is allowed to acccumulate, go low enough to kill the ova of this roundworm.

Surface-soil temperature of 131.9 degrees F. (55.5° C.) were obtained in Kentucky and Minnesota on plots which had no protection from the sun. In cultures under these conditions, ova disintegrated before reaching the infective stage.

Disintegration of ova was greater in sand than in humus soil both in part shade and complete shade during the summer in Minnesota.

In Kentucky no apparent disintegration occurred in clay and sand soil with 42 days' exposure in complete shade during the latter part of July and the month of August.

Laboratory tests indicate that, at a temperature of from 27° to 30° C., a relative humidity of 75 per cent is the lowest in which the ova of this species will complete development.

It is not probable that ascarid ova can utilize atmospheric moisture before it is precipitated. Thus, retardation of development in air which is less than saturated with moisture is caused by loss of water from the ova. When this loss of water is great enough, death from dessication results.

Disintegration of ova in the soil from dessication is the result of the combined effects of a series of interdependent physical factors of which soil type can not be excluded.

PRACTICAL APPLICATION

Recommendations in regard to the reduction of soil infections of ascarid worms, based upon the data given in this report, can only be of a prophylactic nature. It is true here as in any such measure, that the efficiency of the method is determined by how rigidly one adheres to it. Laboratory tests show that the ova of *Toxocara canis* do not undergo development at a temperature under 50° F. Thus, if animal pens are cleaned of all excrement in the late fall, animals are relatively safe from new infections during the winter months, because ova deposited within this period do not develop until the temperature rises the following spring. This practice of thoroly cleaning pens of the remains of broken down animal stools in the late fall, or removing animals at this time to pens which are known not to have infective ova on the floors, should greatly reduce new infections during the winter season. It is true, however, that all ova can not be eliminated from pens that have dirt floors by removing the remains of excrement, as many eggs become mixed with the surface soil. If snow is removed from the pens during the winter months instead of being allowed to accumulate as is the usual practice, there is a very good chance that ova on the floors will be killed by the extremely low temperatures of our northern states. Laboratory tests indicate that the ova of this species are killed at —25° F. The data in this report clearly demonstrate that this temperature will not be attained on the floors of animal pens if they are covered with snow.

An effective recommendation for the reduction of new infections during the summer, and perhaps for a complete sterilization of the soil of all ova near the surface, is to provide sufficient sunlight in animal pens during the summer months. Observations on the longevity of the ova of *Toxocara canis* on the surface of the soil, when exposed to different amounts of sunlight during the summer months, support the validity of this recommendation. It is a general practice among breeders to have pens in the shade. This provides ideal conditions for almost all the ova to develop to the infective stage, and to remain viable for long periods. The present status of these studies indicates that the provision for full sunlight in animal pens during the summer months is one of the most effective means of destroying ascarid ova that are present. This applies to pens that have wood floors as well as to those in which the soil serves as a floor.

LITERATURE CITED

Brown, Harold W.
1927 Studies on the rate of development and viability of the eggs of *Ascaris lumbricoides and Trichuris trichiura* under field conditions. Jour. Parasitol. **14**, No. 1:1-15.

———
1928 Further studies on the longevity of the eggs of *Ascaris lumbricoides* and *Ascaris suum*. Jour. Parasitol. **15**, No. 1:14-22.

Caldwell, Fred C. and Caldwell, Elfreda L.
1928 Preliminary report on observations on the development of ova of pig and human *Ascaris* under natural conditions, and studies of factors influencing development. Jour. Parasitol. **14**, No. 4:254-60.

Cram, Eloise B.
1924 The effects of low temperature and disinfectants on the eggs of *Ascaris lumbricoides*. Jour. Agr. Research **27**, No. 3:167-75.

Davaine, C.
1862 Nouvelles recherches sur le development et la propagation de l'Ascaride lombricoide et du Trichocephale de l'homme. Compt. Rend. Soc. d. Biol. 14:261-65.

Küchenmeister, F.
1857 Manual of animal and vegetable parasites of human body. Vol. 1, p. 311.

Martin, H. M.
1922 The common roundworm of swine (*Ascaris lumbricoides*). Nebr. Agr. Expt. Sta. Circ. 17.

McCulloch, E. C., Graham, Robert, and Carroll, W. E.
1927 Viability of parasitic worm eggs. Illinois Sta. Rept., pp. 91-92.

Otto, G. F.
1929 A study of the moisture requirements of the eggs of the horse, the dog, human and pig ascarids. Am. Jour. Hyg. 10, No. 2, 497-520.

Owen, William B.
1928 Survival of the ova of *Toxocara canis* (*Belascaris marginata*) under field conditions. Jour. Am. Vet. Med. Assoc. 74:58-62.

Raffensperger, H. B.
1927 Viability of *Ascaris suum* ova exposed to weather conditions. Vet. Med. 22:221-26.

Riley, William A., and Owen, William B.
1928 Are *Ascaris* eggs in the soil killed by our northern winters? Am. Fur Breeder 1, No. 3:20.

Scott, J. Allen
1928 An experimental study of the development of *Ancylostoma caninum* in normal and abnormal hosts. Am. Jour. Hyg. 8, No. 2:158-204.

Spindler, L. A.
1929 On the use of a method for the isolation of *Ascaris* eggs from soil. Am. Jour. Hyg. 10, No. 1, 157-64.

Van Verloren Marg. Corn. Eljeren
1854 *Ascaris marginata*. Anteeken, Utrecht. Genootsch. Sect. Nat. en Geneesk, pp. 41-47.

Yoshida, S.
1920 On the resistance of *Ascaris* eggs. Jour. Parasitol. 6, No. 3:132-39.